

## Efficiency of sampling methods to monitor the bacterial contamination of pork carcasses before and after chilling

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### Abstract

The aim of the study was to compare the efficiency of destructive (excision) and non-destructive (swabbing and sponging) sampling methods to enumerate aerobic mesophilic microorganisms, *Enterobacteriaceae* and to detect *Salmonella* on the surface of pork carcasses.

In three slaughterhouses, a total of 720 half-carcasses were sampled before and after chilling. On each half-carcass, four sites were sampled by both destructive and non-destructive techniques, for a respective total surface of 25 and 400 cm<sup>2</sup>.

The dispersion of lognormal distributions describing aerobic colony and *Enterobacteriaceae* counts were not significantly affected by the type of sampling method or by chilling. On the contrary, the mean of bacterial counts was significantly affected by the sampling technique with a better recovery for the destructive method, showing contaminations approximately 1-2 log cfu/cm<sup>2</sup> higher than those obtained with the non-destructive techniques. The same influence was also observed for the detection of *Salmonella* with concentrations decreased by 0.9 and 1.2 log MPN/cm<sup>2</sup> by sponging and swabbing compared to excision.

A significant impact of chilling was observed in two slaughterhouses with bacterial contaminations approximately 1 log cfu/cm<sup>2</sup> lower after chilling, but not for the detection of *Salmonella*. For the non-destructive techniques, the efficiency of sponging to recover microorganisms was higher than swabbing. Complementary tests performed on 120 refrigerated pork meat cuts, with 2 different types of swabs and sponges, confirmed the better recovery with excision followed by sponging and then swabbing (respectively -0.3 and -0.6 log cfu/cm<sup>2</sup>). The recovery differences between the 2 types of swab and sponges tested were about 0.1 log cfu/cm<sup>2</sup>. No impact of the surface sampled by excision of 5, 25 and 100 cm<sup>2</sup> was observed a complementary study for aerobic mesophilic microorganisms, *Enterobacteriaceae*, but a negative effect of increasing surface was observed for the detection of *Salmonella*.

These results reinforce the significance of the sampling technique and stage when monitoring process hygiene criteria.

### Introduction

Monitoring of the bacterial contamination of pig carcasses is an effective way to check the correct application of good hygiene practices during slaughtering. European regulation (EC) No 2073/2005 (Anonymous, 2005) set microbiological criteria including the enumeration of aerobic mesophilic microorganisms and *Enterobacteriaceae* and the detection of *Salmonella* for pig carcasses after dressing and before chilling as process hygiene criteria. These process hygiene criteria help food business operators by indicating an acceptable functioning of the production process or, on the contrary, a breaking in their hygiene procedures.

A relevant bacterial indicator shall thus be correlated with the hygiene practices and, furthermore, shall be reproducible and sufficiently sensitive (level of contamination above the quantification limit of the analytical method). Different sampling methods can be used before microbiological analysis and carcasses

can also be sampled at different stages, particularly after chilling since it can be easier for operators. These protocols can have a significant impact on observed contaminations and therefore on the final decision regarding the control of the process. The aim of this study was to compare the efficiency of destructive and non-destructive sampling methods before and after the chilling of pig carcasses.

## Material and Methods

The samples were obtained in three different slaughterhouses during two days in July 2007. A total of 720 half-carcasses were sampled by two operators before and after chilling. On each half-carcass, four sites (ham, back, belly, and jowl) were sampled by both destructive (excision) and non-destructive (swabbing and abrasive sponging) techniques, for respective total surfaces of 25 and 400 cm<sup>2</sup>. Samples were afterwards diluted in 100 ml of buffered peptone water and plated onto plate count agar for the enumeration of aerobic mesophilic microorganisms and onto violet red bile glucose agar for the enumeration of *Enterobacteriaceae*. The detection of *Salmonella* was performed according the alternative validated SMS method (AFNOR AES-10/4-05/04).

Complementary experiments were carried out with 120 refrigerated pork meat cuts to compare the efficiency of the destructive method compared to two types of abrasive sponges and two types of swabs. The impact of the sampled surface on observed contamination levels for the aerobic mesophilic microorganisms, *Enterobacteriaceae* and *Salmonella* was also studied by sampling by excision pork meat cuts surfaces of 5, 25 and 100 cm<sup>2</sup>.

The distributions of bacterial contamination were assumed lognormal and observed log counts with censored data (enumerations below the quantification threshold) were fitted to normal distributions using the MLE subroutine of MATLAB 7. The concentrations of *Salmonella* were estimated from the proportions of positive detection by assuming a Poisson distribution of bacterial cells on the surface of carcasses and meat cuts.

## Results

The distributions of observed log counts were satisfactorily described by normal distributions and the dispersion of the between-carcasses counts were not significantly affected by the sampling technique (Fig. 1) or by chilling. The mean standard deviations were 0.5 and 1.0 log cfu/cm<sup>2</sup> for aerobic mesophilic microorganisms and *Enterobacteriaceae* distributions, respectively.

On the contrary, the mean of bacterial counts was significantly affected by the sampling technique with a better recovery for the destructive method (Fig. 1).

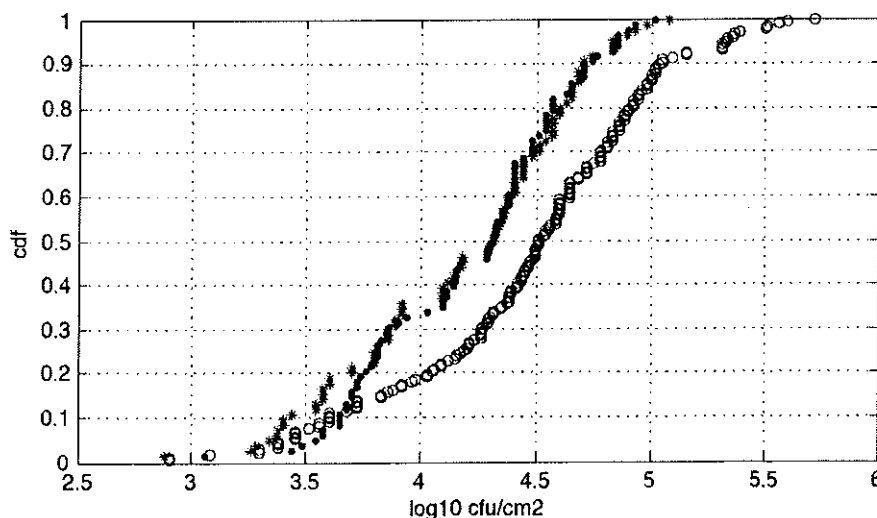


Fig. 1. Cumulative distributions of aerobic mesophilic log counts obtained by excision (○), sponging (●) and swabbing (\*) of pig carcasses in slaughterhouse A.

The contaminations observed with non-destructive methods for aerobic mesophilic microorganisms, *Enterobacteriaceae* and *Salmonella* were respectively approximately 0.5, 1.0, and 1.3 log cfu/cm<sup>2</sup> lower than those obtained with the excision technique (Table 1). Furthermore the non-destructive methods appeared less reproducible than the excision method since results seemed sometimes very dependent on the operator performing the sampling. Differences of more than 0.5 log cfu/cm<sup>2</sup> were for example observed for the enumeration of aerobic mesophilic microorganisms of samples obtained by two operators by sponging and swabbing in the slaughterhouse B (Table 1).

Among the non-destructive methods, the swabbing technique appeared less effective than abrasive sponging for the enumeration of *Enterobacteriaceae* and *Salmonella* with bacterial concentrations 0.6 log cfu/cm<sup>2</sup> lower.

Similar results were observed for the enumeration of *Enterobacteriaceae* of pork meat cuts with contaminations lowered by 0.3 log cfu/cm<sup>2</sup> for sponging and by 0.6 log cfu/cm<sup>2</sup> for swabbing in comparison to contaminations observed by excision.

Table 1. Bacterial contaminations observed on pig carcasses with destructive and non-destructive sampling methods before and after chilling.

Slaughterhouse	Stage	Day	Operator	Aerobic mesophilic microorganisms			<i>Enterobacteriaceae</i>			<i>Salmonella</i>		
				Excision	Sponge	Swab	Excision	Sponge	Swab	Excision	Sponge	Swab
A	H+C <sup>(a)</sup>	1+2	a+b	4.5 (171) <sup>(b)</sup>	4.2 (167)		0.9 (240)	0.1 (120)	-0.6 (120)	-3.2 (240)	-4.0 (120)	-4.4 (120)
B	H	1	a	4.7 (112)	3.3 (88)	4.4 (29)	2.0 (120)	0.8 (60)	0.3 (60)			
		2	b		3.8 (117)					-2.7 (240)	-4.4 (120)	-4.7 (120)
	C	1	a	3.9 (101)	3.3 (88)		1.0 (120)	0.4 (60)	-0.6 (60)			
		2	b		3.8 (117)							
	C	1+2	a+b	4.2 (70)	4.2 (178)		0.2 (119)	0.4 (60)	-0.7 (60)	-2.5 (240)	-3.4 (120)	-3.7 (120)
		1+2	a+b	—			—	-1.0 (60)	-2.0 (60)			

<sup>(a)</sup> H Hot carcass before chilling, C Chilled carcass

<sup>(b)</sup> Mean bacterial contamination in log cfu/cm<sup>2</sup> (number of results).

The impact of chilling was very dependent on the slaughterhouse, no difference in bacterial counts were observed for the slaughterhouse A while a significant impact was observed for aerobic mesophilic microorganisms and *Enterobacteriaceae* in slaughterhouse B and only for *Enterobacteriaceae* in slaughterhouse C (Table 1). When this impact was observed, the bacterial contaminations were lowered by approximately 1 log cfu/cm<sup>2</sup> after chilling.

The sampled surface by excision of pork meat cuts did not affect the bacterial concentration for aerobic mesophilic microorganisms and *Enterobacteriaceae*. However a significant effect was observed for *Salmonella* with a decreasing concentration with increasing sampled surface (Table 2).

Table 2. Bacterial contaminations observed on pork meat cuts with excision of different surfaces.

Experiment	Surface (cm <sup>2</sup> )	Aerobic mesophilic microorganisms	<i>Enterobacteriaceae</i>	<i>Salmonella</i>
#1	5	5.4 (22) <sup>(a)</sup>	2.1 (69)	<-1.8 (50)
	25	5.5 (20)	2.3 (70)	-2.1 (50)
	100	5.4 (22)	2.2 (70)	-3.2 (50)
#2	5	—	2.0 (30)	-1.5 (198)
	25	—	1.8 (30)	-2.0 (198)
	100	—	1.8 (30)	-2.4 (198)

<sup>(a)</sup> Mean bacterial contamination in log cfu/cm<sup>2</sup> (number of results).

A decrease of 0.5-1 log MPN/cm<sup>2</sup> was observed when the sampled surface was increased from 25 cm<sup>2</sup> to 100 cm<sup>2</sup>.

## Discussion

Excision method appeared as the most effective method to recover microorganisms on the surface of meats followed by abrasive sponging and swabbing. The efficiency of excision to recover microorganisms in comparison to non-destructive methods has been previously reported by many studies (Snijders et al, 1984 ; Yu et al, 2001 ; Ghafir et Daube, 2008). The sensitivity of bacterial indicators can thus be significantly affected when non-destructive techniques are used and furthermore the reproducibility of these techniques seemed sometimes poor and dependent on the operator performing the sampling.

Complementary tests (data not shown) performed on 120 refrigerated pork meat cuts, with 2 different types of swabs and sponges, confirmed the better recovery with excision followed by sponging and then swabbing (respectively -0.3 and -0.6 log cfu/cm<sup>2</sup>). The recovery differences between the 2 types of swabs and sponges tested were low (about 0.1 log cfu/cm<sup>2</sup>), whereas Pearce et Bolton (2005) found a difference of 0.5 log between Polyurethane and Cellulose acetate sponges.

Regarding the impact of chilling on bacterial contamination, it seemed difficult to conclude univocally and the effect seemed very dependent on the slaughterhouse. Yu et al (2001) reported that numbers of all bacteria present on the post-chill carcasses were substantially lower than on the pre-chill carcasses. Individual studies should be carried out in each slaughterhouse to justify the sampling of chilled or hot carcasses.

The size of the sampled surface had no effect on enumeration of bacterial indicators but an unexpected effect was observed for the detection of *Salmonella*. Indeed, the percentage of positive results weakly increased in comparison to the increasing sampled surface resulting in estimated concentrations lower for large sample sizes in comparison to small ones. This effect could be linked to a decreasing sensitivity of bacteriological analysis methods when the sample size increases and the initial dilution factor is lowered.

## Conclusion

These results reinforce the significance of sampling techniques to monitor the bacterial contamination of pig carcasses or pork meat cuts. The microbiological limits distinguishing satisfactory contaminations from unsatisfactory results must be set by taking into account the sampling technique used and the stage of the process. It was also observed that this setting must also take into account the influence of the sampling technique on the performances of analytical methods used afterwards.

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